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# BIOLOGICAL BULLETIN

## ON THE PRESENT STATUS OF THE CHONDRIOSOME-PROBLEM.[1]

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Before I come to the subject of this address, I want to make a short incursion into the domain of nomenclature. As I recently pointed out, the word "mitochondrium" designates a body of granular form. Since those who use it as a general denomination have in mind certain bodies which very often appear as filaments, they are led to speak of "filamentous mitochondria," something which is inconceivable. Besides, using a term in a new sense adds greatly to the confusion already existing in the nomenclature, much more than the use of a new term. In 1915 I reverted to the use of the word "chondriosome," for although there may be some objection to it, in the present state of things it has two distinct advantages—one, that it does not preclude anything as to the form of the bodies which it is supposed to designate; the other, that it is already extensively used, so that its further adoption would bring us very near to that much-to-be-desired unification in our nomenclature.

Speaking of the present status of the chondriosome-problem, I have to speak of what has been done in this particular field of cytological research, and also of what is still to be done. There is much to say on either topic, since the number of observations published on chondriosomes is enormous and since an agreement on some the most fundamental questions is as yet far from being reached. Owing to lack of time, however, my account must necessarily be confined to a few essential points.

Beginning with what has been accomplished, one of the most gratifying results for the cytologist who works in that field is the

[1] Lecture delivered at Woods Hole Marine Biological Laboratory, Mass., July 23, 1918.

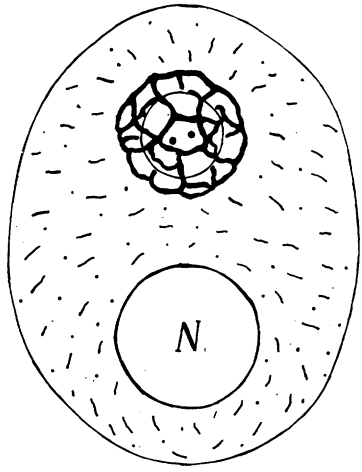
following. After the publication of Benda's somewhat complicated method, it was a common opinion that what that method brings into evidence is only a distorted appearance of the cellular structures. Thus, however, were disregarded a number of observations, some of which belong to the earliest period of cytological investigation: I shall limit myself to mention von Brunn's observations on the formation of the spiral filament in the spermatozoön of the mouse, von la Valette St. George's work with dahlia-violet applied in vivo, Michaelis's discovery of the peculiar properties of the janus-green.[1] Quite lately the chondriosomes have been extensively studied in the living cell, especially in tissue cultures: prominent investigators in this field are W. H. and M. R. Lewis. These recent observations have confirmed what the old ones already taught—that chondriosomes are not artefacts and that they appear in the fixed and stained preparations very much the same as they are in the living cell.

Another important result of the recent studies on chondriosomes is a simplification in our conception of the morphology of the protoplasm. When Benda first described his "mitochondria" (at that time, he was convinced that the granular form was the rule), he thought he had discovered a new cellular organule. The question soon arose: what is the relationship between these organules and other constituents of the cell, such as Flemming's fila, Maggi's plastidules, Altmann's bioblasts, Boveri's archoplasmic granules, etc.? Is there any relation between these bodies or are they entirely different? In the last supposition, the structure of the cytoplasm appeared as something so complicated that many, especially under the influence of Fischer's experiments, cleared the field by designating as an artefact any structure which appeared after fixation. We know now, principally as the result of Meves's exertions, that all these elements are one and the same thing. Flemming's filaments (at least those which he described in the resting cell, in contradistinction to the mitome of the dividing cell), Maggi's plastidules found by that author and by the brothers Zoja, his pupils, in practically

[1] Probably the reason why janus-green has until lately been so little in favor among cytologists is that a number of dyes delivered under that name are either some other product, or a substance mixed with impurities, which may be toxic.

all tissues, Altmann's bioblasts whose discovery unfortunately led their author to theoretical considerations which threw disrepute on his observations, Boveri's archoplasmic granules which in the dividing egg of *Ascaris megalocephala* surround the centrosphere,—all these things are identical with Benda's mitochondria and with our chondriosomes. We know further that the morphology of that same substance in the different cells, or even in a given cell at different periods of its life, is subject to great variations. Thus the controversy between the partisans of an exclusively filamentous structure of the protoplasm and those who believed only in granules, has come to an end. The morphological variations of chondriosomes in one cycle of life are especially conspicuous in the seminal cells of many invertebrates. There, in many species, the chondriosomes of the spermatogonia are granules; in the spermatocytes, the granules flow together and build up filaments and finally in the spermatid, a further coalescence very often takes place, the result of which is the formation of a single, compact, bulky body, well-known as von la Valette St. George's "Nebenkern." A reverse process is also observed. To return to an example already given, Boveri's archoplasmic granules in the dividing egg of *Ascaris* are derived from bodies which have, in the young ovocyte, a filamentous form. One should be warned however against reuniting all protoplasmic structures in one single group, as some have done. As far as we know, there is no relationship between the chondriosomes and the idiosome, nor between the chondriosomes and most of the elements designated as Golgi's apparatus. It is becoming more and more clear in my opinion that the latter denomination should be reserved to that element which in most cells, especially in young cells and during the period of rest, is closely connected with the idiosome and which has been described under various names, such as Centrophormien, Zentralkapseln, formazioni periidiozomici, idioectosome, etc. If I had to express in a schema the structure of a young resting cell, I would propose this one, (s. next p.) which in my opinion, corresponds to the present state of our knowledge. It should be added here that, for the majority of investigators, including myself, the chondriosomes belong to the cytoplasm. Quite recently, the question of their

nuclear origin has been brought up again by Schreiner. Taking for granted that Schreiner's description be accurate, nothing proves however that his interpretation be correct, for the process described as an expulsion of substance from the nucleus might just as well be the reverse. And even if we accept Schreiner's interpretation, we find that the question of the origin of the




*N*, nucleus; above, idiosome with centrioles, surrounded by the apparatus of Golgi. In the protoplasm, chondriosomes.

chondriosomes has really not been touched, since chondriosomes are present in all cells before the process which Schreiner describes takes place.

Finally, the tremendous amount of work published in recent years has revealed the presence of chondriosomes in practically all cells, animal and vegetal. The only animal cells that constitute an exception are the superficial cells of the epiderm: that is, cells whose protoplasm is entirely differentiated and transformed into a new substance. Whether chondriosomes exist in the adult red blood-corpuscle might perhaps be questioned also. It is not impossible that the presence of hæmoglobin, which is precipitated by the fixing reagents, be responsible for the negative results obtained by most authors. But, one may well ask, how can the cytologist decide whether he is in presence of chondriosomes or not? A number of criteria may be applied

in this connection: the behavior towards preserving reagents and dyes; or better still, the evolution of a given cell-constituent, as for instance in the seminal cells; or the continuity of a given structure from the embryonic cell to the adult stage. If however our objector means to say that it may be difficult in a given case to decide whether or not a certain structure is a chondriosome, then I heartily agree with him, but I fail to see therein a reason for general scepticism. Rather it should prove a stimulus for further investigation. It should not be forgotten that the chondriosomes are by no means the only structures that may be hard to identify in certain instances. Indeed, sometimes we have great difficulty in recognizing a nucleus. Take for example the controversy on the so-called Blochman's nuclei in the egg of certain insects, or (as a personal illustration) the divergence of opinion between Retzius and myself as to what part of the spermatozoön of *Ciona* is the nucleus. In this very field (spermiogenesis) the same difficulty is met with constantly when we attempt to study atypical forms.

I come now to the second part of this address: what is still to be done in the investigation of the chondriosomes? Since brevity is essential, I shall limit myself to the consideration of two questions, both of great importance *i. e.*, the rôle played by the chondriosomes in the process of differentiation and their rôle in fertilization. Meves was the first to express the opinion that the chondriosomes of the embryonic cell represent an indifferent material, susceptible of various differentiations in the different tissues. Since that time a number of papers supporting this idea have been published, of which I shall only mention those on the development of the collagenous fibrils (Meves), of neurofibrils (Hoven) and of myofibrils (Duesberg). The same idea is also and very strongly supported by a number of observations concerning the processes of differentiation in vegetal cells. My own observations on myofibrillogenesis have found confirmation in different quarters and so far no specific criticism has been expressed. On the other hand, the conclusions concerning the chondriosomal origin of the neurofibrils and of the collagenous fibrils have been criticized, respectively by Cowdry and M. R. Lewis. I frankly admit that Cowdry's arguments are worth

being very carefully considered, but I should perhaps be allowed at the same time to venture the question whether there is really such a problem as the origin of neurofibrils; in other words, whether the neurofibrils really exist in the living cell. As to the fibrils of the connective tissue, a detailed criticism of M. R. Lewis's paper is out of place here, but I want however to point out that little effort has been made by that author to demonstrate that the fibrils appearing in her tissue-cultures are collagenous fibrils. Yet, I gather from Baitsell's observations that great care should be taken in interpreting such fibrillar structures. Personnally, I have not found in my recent investigations on development, normal and regenerative, any reason for changing my mind. I can clearly see however, how interesting it would be to apply the experimental method to the solution of these problems: experiments on regeneration of tissues, on centrifuged eggs, on the behavior of cells under abnormal and pathological conditions and under the influence of poisonous substances, etc., a field which so far has been explored very little.

The second question I want to consider is this: are the chondriosomes an idioplasmic substance? This opinion was first expressed by Benda. It found strong support in Meves's discovery of chondriosomes in all embryonic cells and later in that author's description of the behavior of the male chondriosomes in *Ascaris* during fertilization. The question is so important that it should be considered with greatest care and at some length.

At the basis of the hypothesis of the idioplasmic nature of the chondriosomes is the idea in which the protoplasm plays a rôle in the transmission of hereditary characteristics. This opinion has always been that of a number of biologists, although a minority, and it has been supported by experimental researches, namely by the well-known experiments made by Godlewsky. The question then arises: what part of the spermatozoön represents the protoplasmic idioplasm? It cannot be the axile filament, which is not constant and is obviously an organ of motility; it cannot be the headcap, nor the acrosome, since these are also inconstant, in some cases (marsupials) only transitory parts of the spermatozoön; nor do I see that any argument can be brought forth in favor of the idioplasmic nature of the centrioles, or of

the small amount of protoplasmic ground-substance which, in the mammalian spermatozoön, forms a thin covering over the head and the proximal part of the tail. But there is another constituent which the recent studies have shown to be constant in all spermatozoa of all species, from the lowest to the highest groups. These are the chondriosomes. The same studies have shown also that the chondriosomes can assume in the ripe spermatozoön a shape and location which vary greatly in the different species. One used to say that the chondriosomes form a sheath around the "middle-piece" of the spermatozoön. This is a very loose, and in many cases an entirely incorrect statement. Loose, since under the term "middle-piece" we designate parts that have nothing in common: there is no homology whatsoever between the so-called "middle-piece" of the spermatozoön of an echinoderm, of a selachian (or an amphibian) and of a mammal. In many cases it is also an incorrect statement. In a number of higher vertebrates, especially in mammals, the chondriosomes actually form a sheath around what is customarily called the "middle-piece" of the spermatozoön; at the same time, the shape of that sheath varies a good deal, from isolated granules to discs or a spiral filament. In lower vertebrates (in many fishes) and in a great number of invertebrates (many molluscs, certain worms, etc.), the chondriosomes are represented by a small number of granules located on the posterior part of the head and surrounding the origin of the tail. A third type often met with, especially in insects, is represented by a long, thin sheath, which envelops the axile filament; this sheath is derived from the so-called "Nebenkern," which usually breaks in two, the pieces afterwards becoming elongated as the tail grows. But besides these three main types there exists also an infinity of other forms. Quoting at random I would mention the spermatozoa of *Ascaris*, *Phallusia*, *Ciona*, of many worms, crustacea, molluscs, etc. It is consequently impossible, in the presence of the ripe spermatozoön only, to decide what part of it is formed by the chondriosomes[1] and only a careful study of spermiogenesis can solve the problem. Indeed such a study should be undertaken

[1] This is a reason why I cannot, without further investigation, consider F. R. Lillie's observations on fertilization in *Nereis* as demonstrating that in that species the male chondriosomes do not penetrate into the egg.

every time one has to deal with atypical forms of spermatozoa as a preliminary to the study of fertilization.

The chondriosomes being a constant constituent of the spermatozoön, have we any clue as to their significance? Opinions differ on this point. Benda is responsible for a hypothesis according to which the chondriosomes are in some way connected with the property of motility. This hypothesis reappears occasionally, and in my opinion very unfortunately, since it is not supported by anything and is contradicted by a number of facts. Of course, one can well imagine how such an idea might be suggested by the appearance of the spiral filament of certain spermatozoa, just as a rather naïve theory of muscular contraction was once built upon the conception that the myofibrils were shaped like a spring. But I fail utterly to see how other forms of the chondriosomal sheath, such as the small granules existing in so many lower vertebrates and in many invertebrates, could suggest in any way a relation with the motility of the spermatozoön. Furthermore, Benda's hypothesis is in contradiction to Meves's experiments of merotomy on spermatozoa and to the presence of chondriosomes in those spermatozoa that make no active movements, such as the spermatozoa of decapods. The same facts disagree also with the idea expressed by Regaud, for whom "le chondriome du spermatozoide est moins un matériel héréditaire qu'une partie de la cellule jouant un rôle actuel de fixation et de concentration des substances ambiantes destinées à être consommées lors de la contraction du filament axile."

Another opinion was expressed by Koltzoff. This author considers the chondriosomes as playing the rôle "einer formbildenden Substanz." While this is apparently true for certain spermatozoa he studied, for decapods for instance, I would deny that Koltzoff's interpretation has any general value. On physical grounds it would seem impossible that bodies which are entirely enclosed in a cell, without coming in contact with its surface, could have anything to do with the form of that cell. To return to the type of spermatozoön so common among lower vertebrates and invertebrates, is it possible to imagine that these granules have anything to do with determining the form of the spermatozoön? The answer can only be a negative one.

Finally we come to the hypothesis according to which the chondriosomes represent the idioplasm contained in the protoplasm of the seminal cells. We must find out first what becomes of the male chondriosomes in the process of fertilization. Although one of the first studies of this process, that made on *Ascaris megalocephala* by van Beneden, showed that the whole spermatozoön may enter the egg, the great majority of biologists have accepted the dogma of the monopoly of the nucleus as carrier of the idioplasm. The reasons for that attitude are obvious. First, in most cases, only the nucleus of the spermatozoön was detected in the egg; further, investigators were quite naturally hypnotized by the beautiful karyokinetic figures which they saw formed in the fertilized egg at the expense of the pronuclei. Yet, after van Beneden, a number of other authors (Kostanecki, Nekrassof, Lams, etc., in invertebrates; Fick, Nicolas, Michaelis, Van der Stricht, etc., in vertebrates) have demonstrated that other parts of the spermatozoön are also carried into the egg. The hypothesis in which the chondriosomes represent an idioplasmic substance has given a renewed interest to these observations which had been considered as exceptional cases, and recent observations, made under the impulse of the same hypothesis, are likely to lead us to a reconsideration of our conception of the morphology of fertilization. I need only mention in this connection the case of the sea-urchin. It was generally accepted that only the nucleus and the so-called "middle-piece" penetrate into the egg and that the tail is left without. Recent observations, however, made with special care and a more refined technique, have shown that the tail also enters. Let us not forget, however, that the crucial point to determine is not whether the tail, but whether the chondriosomes are carried into the egg. As I pointed out when speaking of the structure of the spermatozoön, these questions have no connection. One can readily conceive of a type of spermatozoön in which only the head would penetrate, and yet the chondriosomes would enter the egg at the same time. Such a type exists, as a matter of fact, in a number of invertebrates, for instance the ascidians *Phallusia* and *Ciona*.[1]

[1] In these two species, however, the tail also is found in the egg.

We have observations, consequently, showing that the tail of the spermatozoön, or part of it, penetrates into the egg. We come now to the cases where the chondriosomes have actually been found in the egg. Most of these cases have been described recently under the impulse of the chondriosomal theory, and, considering the short time intervening since the publication of that theory, their number is already quite impressive. The actual penetration of chondriosomes into the egg was seen long ago, however, by L. and R. Zoja in *Ascaris*, and later by Vander Stricht in the bat. To these observations have been added, during the last seven years, a number of others: on the sea-urchin, *Phallusia*, *Mytilus* and *Filaria* (Meves) and on *Ciona* (Duesberg). Thus we already have observations on a number of invertebrates (worms, ascidians, echinoderms and molluscs) and vertebrates (mammals and amphibians—for there is no doubt from the observations of Fick and Michaelis that in *Triton* and *Axolotl* also the male chondriosomes are carried into the egg).

While things would thus appear to be in perfect agreement with the theory according to which the chondriosomes are idioplasm, a difficulty, and a very serious one indeed, has arisen. First, Vander Stricht and his pupils found in mammals that the chondriosomal part of the spermatozoön (in the bat, the spiral filament) passes unchanged into one of the first two blastomeres. Similarly in the sea-urchin, the chondriosomal middle-piece is found in one of the daughter-cells after the first division (Meves). So far the difficulty could be met, for there is some reason to believe that the two first blastomeres of the mammalian egg are not equivalent, one of them possibly forming the trophoblast only, the other the embryo, and a similar hypothesis could be formulated concerning the sea-urchin, since the adult is formed of parts only of the original embryo. Meves supposed that the male chondriosomal substance would go over into these cells which build up the definite animal. His further investigations on the fate of the middle-piece, up to 32 blastomeres, show that its fate is variable and that certainly not all cells of the young sea-urchin will receive male chondriosomal substance.

Here lies the real difficulty, and its weight seems overwhelming. I would however warn against too hasty conclusions. A number

of considerations: the constancy of the chondriosomes in the spermatozoön, the lack of a satisfactory hypothesis as to their significance[1] and the fact that in a number of adequately investigated cases they have been found to penetrate into the egg, render, in my opinion, further research necessary before a decision can be reached. The study of fertilization is indeed a difficult one, especially the investigation of such minute constituents of the spermatozoön amid the huge mass of the egg, but it is of such fundamental importance that the investigator will find in it his reward.

[1] Since the middle piece of the spermatozoön does not change its shape nor its volume in the egg of the sea-urchin, it can apparently not be considered as having the value of a chemical factor, such as postulated by Loeb in his theory of fertilization.